REMARKS

Claims 46-51, 53-57 and 60-64 were pending in the present application prior to the present amendment. New claims 65-69 have been added and Claims 46 and 64 have been amended. The Examiner found that Claims 53-57 and 60-64 were allowable, but 46-51 and 64 were rejected. Applicants believe that the Examiner intended to state that 60-63 are allowable and that 64 is rejected. If this supposition is incorrect, Applicants respectfully request clarification from the Examiner.

Applicants point out that Mohan et al. (a complete copy of this reference is appended for the Examiner's convenience) does not disclose the preservative properties of quercetin. On the contrary, the final sentence of the "Materials and Methods" paragraph reads: "The eye drops were prepared fresh every 3rd day in 0.5% methylcellulose[.]" As observed by the Examiner the formula contains no preservative. Applicants respectfully suggest that is not evidence that Mohan knew of the preservative properties of flavonoids; rather, the lack of an added preservative was why the authors specified that the drops be made up fresh every third day. If Mohan et al. had been aware of the preservative properties of quercetin, the three day limit on drop life would not have been mentioned.

Applicants also appreciate the Examiner's point that an open claim such as Claim 46 claiming a range of 10 to 10,000 ppm could be considered anticipated by Mohan et al.'s use of 0.5% (5,000 ppm) of one of the claimed agents. Therefore, quercetin has been removed from Claims 46 and 64 and is now the subject of a newly presented independent Claim 69 wherein the concentration of quercetin is below that specified by Mohan et al. Therefore,

new Claim 69 is not anticipated by Mohan et al. and, Applicants respectfully submit, is patentable over the art of record.

Now that Claims 46 and 64 no longer call out quercetin, they, as well as those claims dependent upon them, are allowable. In view of the foregoing, it is respectfully submitted that the application is in condition for allowance. Reexamination and reconsideration of the application, as amended, are requested.

If for any reason the Examiner still finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles telephone number (310) 734-5200 to discuss the steps necessary for placing the application in condition for allowance.

You are hereby authorized to charge any fees due and refund any surplus fees to our Deposit Account No. 50-2567.

Respectfully submitted,

REED SMITH CROSBY HEAFEY

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Copy of Mohan et al.

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Anti-cataract effect of topical quercetin and myricetin in galactosemic rats

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The role of aldose reductase (AR) in the causation of diabetic cataract is now well established [1]. One major finding to support the role of AR in sugar cataract is the prevention and delay in the progression of cataract development by certain AR inhibitors [2–4]. Flavonoids have been reported to be potent inhibitors of lens AR [5, 6]. Of these, quercetin and myricetin have been shown to be more potent in comparison with others [7]. So far only limited attempts have been made to evaluate the efficacy of these flavonoids as anti-cataract agents [8]. In the present study the effect of topical quercetin and myricetin in galactosemic cataracts was assessed.

Materials and methods: Galactose cataracts were induced in young albino rats (60–80 g b.wt) maintained in standard laboratory conditions. Animals were fed a diet containing 30% galactose and were randomly divided into three groups of 12 rats each. Group 1 served as control. In group 2 and 3 animals, quercetin (0.5%) and myricetin (0.1%) eye drops were applied, respectively. The eye drops were prepared fresh every 3rd day in 0.5% methylcellulose and were applied three times a day in both the eyes of the experimental animals while the vehicle was applied in the control group.

The stages of cataract (1 to 5) were graded according to the classification of Sippel [9] as described below:Stage 1A, thin band of vacuoles in the periphery;Stage 1B, vacuoles increase and occupy one third of the lens in anterior cortex;Stage 1C, vacuoles occupy two thirds of the lens;Stage 2, vacuoles now reach the centre of the lens and liquefication of vacuoles begins; Stage 3, vacuoles have liquefied and a uniform opalescence develops; Stage 4, nuclear opacification begins; Stage 5, total involvement of lens.

The eyes were examined every alternate day by the slit lamp retro-illumination technique. Morphological changes in drug treated groups were compared with the control group. Percent reduction in the numbers of animals developing cataracts or the delay in reaching the different stages of cataractogenesis was considered as the anti-cataract effect.

Six rats from each group were killed by decapitation on the 10th day of galactose feeding and their lenses removed for dulcitol assay. In the remaining animals the diet and drug treatment schedule were continued for a further 30 days when all the animals were killed and their lenses dissected out for an assessment of their dulcitol content by the method of West and Rapoport [10]. Blood glucose and galactose levels were measured by the method of Hultman [11].

Results and discussion: The anti-cataract action of quercetin and myricetin was assessed by comparing the percentage of eyes developing cataract and the stage-wise progression of cataract formation in drug and vehicle treated eyes when subjected to cataractogenic challenge. The results are shown in Table 1. The onset and cataract development were significantly delayed in the eyes treated topically with quercetin or myricetin. On the 4th day, 50% of the quercetin treated eyes and 75% of the myricetin treated eyes were normal whereas 100% of the eyes in the control group had developed a thin band of peripheral opacities (stage 1A). Besides delay in the onset, development of cataract appeared to be slower in the treated eyes. By the 30th day, 100% of eyes in the control group had developed nuclear opacity (stage 4) while the eyes in the treated groups had progressed to stage 2 only. The stages of eyes in all the three groups remained stationary till the 40th day. Statistical analysis by χ^2 test indicated that the differences were highly significant ($p < 0.001$).

Table 1: Anti-cataract effect of topical quercetin and myricetin on the development of cataracts in rats (number of eyes in each group = 24)

Groups	Stage of cataract	Number of eyes on different days of galactose feeding					
		4	8	14	19	30	40
Control	N[a]	–	–	–	–	–	–
	1A	24	–	–	–	–	–
	1B	–	24	2	–	–	–
	1C	–	–	22	–	–	–
	2	–	–	–	24	–	–
	3	–	–	–	–	–	–
	4	–	–	–	–	24	24
	5	–	–	–	–	–	–
Quercetin (0.5%)	N	12**	–	–	–	–	–
	1A	12**	24**	8*	–	–	–
	1B	–	–	16**	16**	–	–
	1C	–	–	–	–	–	–
	2	–	–	–	8**	24**	24**
	3	–	–	–	–	–	–
	4	–	–	–	–	–	–
	5	–	–	–	–	–	–
Myricetin (0.1%)	N	18**	–	–	–	–	–
	1A	6**	24**	6*	–	–	–
	1B	–	–	18**	–	–	–
	1C	–	–	–	24**	–	–
	2	–	–	–	–	24**	24**
	3	–	–	–	–	–	–
	4	–	–	–	–	–	–
	5	–	–	–	–	–	–

[a]N = normal lens. In comparison with control, $p <$: *0.01; **0.001.

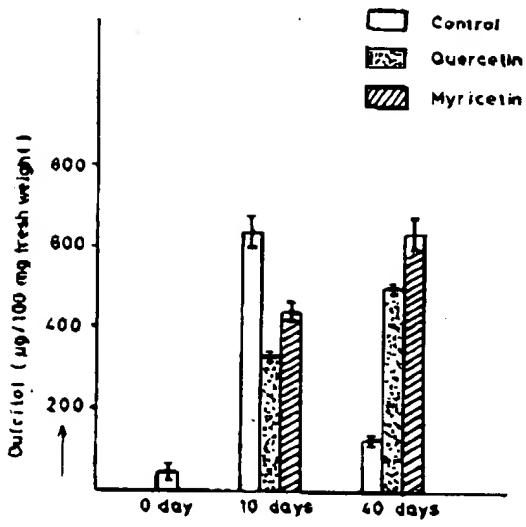

Figure 1: Changes in dulcitol content in controls and in quercetin or myricetin treated lenses at different days of galactose feeding (means ± SEM).

Biochemical studies showed that on the 10th day the level of dulcitol, the polyalcohol product of galactose, was significantly higher in the lenses of the control group as compared with the quercetin and myricetin treated groups ($p < 0.001$, $p < 0.05$, respectively). However, on the 40th day when the control lenses were in stage 4 of cataract, the dulcitol content was found t be less than that in the treated lenses (Figure 1) owing to excessive hydropic swelling and rupturing of lens fibres in the untreated eyes. On the same day, early stages of cataract and higher levels of dulcitol in the treated groups suggested a slower progression of cataract.

Blood glucose and galactose levels were found to be elevated in all the three groups; however, the difference was not significant. No ocular toxicity was observed during the study period. It is concluded that quercetin and myricetin may prove to be potential anti-cataract agents.

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